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(54) Title: ACETYL-COA-CARBOXYLASE FROM CANDIDA ALBICANS

(57) Abstract

The Acetyl-COA-carboxylase (ACCase) gene from *Candida albicans*.

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ACETYL-COA-CARBOXYLASE FROM CANDIDA ALBICANS

The present invention relates to Acetyl-COA-carboxylase (ACCase) genes from *Candida Albicans* (C. albicans) and methods for its expression. The invention also relates to novel hybrid organisms for use in such expression methods.

C. albicans is an important fungal pathogen and the most prominent target organism for antifungal research. ACCase is an enzyme of fatty acid biosynthesis and essential for fungal growth and viability. Inhibitors of the ACCase enzyme should therefore be potent antifungals. The ACCase proteins in all organisms are homologous to each other but they also differ significantly in the amino acid sequence. Because selectivity problems (for example fungal versus human) it is extremely important to optimise potential inhibitor leads directly against the target enzyme (C. albicans) and not against a homologous but non-identical model protein, for example from Saccharomyces cerevisiae (S. Cerevisiae).

We have now successfully cloned the ACCase gene from C. albicans (hereinafter referred to as the C. Albicans ACC1 gene) and elucidated its full length DNA sequence and corresponding polypeptide sequence, as set out in Figures 4 and 5 of this application respectively. The coding DNA sequence of the C. Albicans ACC1 gene is 6810 nucleotides in length and the corresponding protein sequence is 2270 amino acids in length. As will be explained below there are two forms of the C. Albicans ACC1 gene, the above numbers relate to the longer version, Met1.

Therefore in a first aspect of the present invention we provide a polynucleotide encoding a C.albicans ACCase gene, in particular the (purified) C. albicans ACC1 gene as set out in Figure 5 hereinafter. It will be appreciated that the polynucleotide may comprise any of the degenerate codes for a particular amino acid including the use of rare codons. The polynucleotide is conveniently as set out in Figure 4. It will be apparent from Figure 4 that the gene is characterised by the start codons Met1 and Met2 (as indicated by the first and second underlined atg codons, hereinafter referred to as atg1 and atg2 respectively). Both forms of the gene starting from Met1 and Met2 respectively are comprised in the present invention. The invention further comprises convenient fragments of any one of the above sequences.

Convenient fragments may be defined by restriction endonuclease digests of sequence, suitable fragments include a full length C. Albicans ACC1 gene (starting with Met1 or Met2) flanked by unique StuI (5'-end)-NotI (3'-end) restriction sites as detailed in Figure 6.

We also provide a polynucleotide probe comprising any one of the above sequences or fragments together with a convenient label or marker, preferably a non-radioactive label or marker. Following procedures well known in the art, the probe may be used to identify corresponding nucleic acid sequences. Such sequences may be comprised in libraries, such as cDNA libraries. We also provide RNA transcripts corresponding to any of the above C. Albicans ACC1 sequences or fragments.

In a further aspect of the invention we provide a C. albicans ACC1 enzyme, especially the ACC1 enzyme having the polypeptide sequence set out in Figure 5, in isolated and purified form. This is conveniently achieved by expression of the coding DNA sequence of the C. Albicans ACC1 gene set out in Figure 4, using methods well known in the art (for example as described in the Maniatis cloning manual - Molecular Cloning: A Laboratory Manual, 2nd Edition 1989, J. Sambrook, E.F. Fritsch & Maniatis). As indicated for Figure 4 above, the enzyme is characterised by two forms Met1 and Met2. Both form of the enzyme are comprised in the present invention.

The C. Albicans ACC1 enzyme of the present invention is useful as a target in biochemical assays. However, to provide sufficient enzyme for a biochemical assay for C. Albicans ACC1 (for example, for a high throughput screen for enzyme inhibitors) this has to be purified. Two major constraints impair this purification.

1) any new organism will necessitate deviation from published procedures because it will differ in its lysis and protease activity. C. albicans is known to express and secrete many aspartyl proteases.

2) The expression of C. Albicans ACC1 is very low and satisfying purification results can only be achieved if the enzyme is overexpressed.

We have now been able to overcome these problems by controlled overexpression of the C. albicans ACC1 in a Saccharomyces strain. This means that subsequent purification of the enzyme may then for example follow published procedures.

Therefore in a further aspect of the present invention we provide a novel expression system for expression of a C. albicans ACC1 gene which system comprises an S. cerevisiae host strain having a C. albicans ACC1 gene inserted in place of the native ACC1 gene from S. Cerevisiae, whereby the C. albicans ACC1 gene is expressed. Preferred S. cerevisiae strains include.JK9-3Daα and its haploid segregants.

The C. albicans ACC1 gene is preferably over-expressed relative to that as may be achieved by a C. albicans wild type strain, ie under the control of its own ACC1 promoter. Whilst we do not wish to be bound by theoretical considerations, we have achieved approximately 14 fold over-expression relative to the wild-type host S. cervisiae strain JK9-3D. This may be achieved by replacing the C. albicans promoter in the expression construct by a stronger and preferably inducible promoter such as the S. cerevisiae GAL1 promoter.

Controlled overexpression is used to improve expression of a C. albicans polypeptide relative to expression under the control of a C. albicans promoter. In addition using procedures outlined in the accompanying examples we have been able to isolate a fully functional C. albicans ACC1 gene as determined by 100% inhibition by SoraphenA.

The novel expression system is conveniently prepared by transformation of a heterozygous ACC1 deletion strain of a convenient S. cerevisiae host by a convenient plasmid comprising the C. albicans ACC1 gene. Transformation is conveniently effected using methods well known in the art of molecular biology (Ito et al. 1983).

The plasmid comprising the C. albicans ACC1 gene and used to transform a convenient S. cerevisiae host represents a further aspect of the invention. Preferred plasmids for insertion of the C. Albicans ACC1 gene include YEp24, pRS316 and pYES2(Invitrogen).

The heterozygous ACC1 deletion strain of a convenient (diploid) S. cerevisiae host is conveniently achieved by disruption preferably using an antibiotic resistance cassette such as the kanamycin resistance cassette described by Wach et al (Yeast, 1994, 10, 1793-1808).

The expression systems of the invention may be used together with, for example cell growth and enzyme isolation procedures identical to or analogous to those described herein, to provide an acetyl-COA-carboxylase (ACCase) gene from C.albicans in sufficient quantity and with sufficient activity for compound screening purposes.

In a further aspect of the invention we provide the use of an acetyl-COA-carboxylase (ACCase) gene from C.albicans in assays to identify inhibitors of the polypeptide. In particular we provide the their use in pharmaceutical or agrochemical research.

As presented above the C. albicans ACC1 enzyme may be used in biochemical assays to identify agents which modulate the activity of the enzyme. The design and implementation of such assays will be evident to the biochemist of ordinary skill. The enzyme may be used to turn over a convenient substrate whilst incorporating/losing a labelled component to define a test system. Test compounds are then introduced into the test system and measurements made to determine their effect on enzyme activity. Particular assays are those used to identify inhibitors of the enzyme useful as antifungal agents. By way of non-limiting example, the activity of the ACC1 enzyme may be determined by (i) following the incorporation (HCO_3, Acetyl-CoA) or loss (ATP) of a convenient label from the relevant substrate (T.Tanabe et al, Methods in Enzymology, 1981, 71, 5-60; M. Matasuhashi, Methods in Enzymology, 1969, 14, 3-16), (ii) following the release of inorganic phosphate from ATP (P. Lanzetta et al, Anal. Biochem. 1979, 100, 95-97), or (iii) following the oxidation of NADH in a coupled assay, for example using either fatty acid synthetase or pyruvate kinase/lactate dehydrogenase enzymes. Convenient labels include carbon14, tritium, phosphorous32 or 33.

Any convenient test compound(s) or library of test compounds may be used. Particular test compounds include low molecular weight chemical compounds (molecular weight less than 1500 daltons) suitable as pharmaceutical agents for human, animal or plant use.

The enzyme of the invention, and convenient fragments thereof may be used to raise antibodies. Such antibodies have a number of uses which will be evident to the molecular biologist of ordinary skill. Such uses include (i) monitoring enzyme expression, (ii) the development of assays to measure enzyme activity and precipitation of the enzyme.

In addition we provide antisense polynucleotides specific for all or a part of an ACC1 polynucleotide of the invention.

The invention will now be illustrated but not limited by reference to the following Table, Example, References and Figures wherein:

Table 1 shows the comparative properties of native and recombinant acetyl-CoA carboxylase enzymes

Figure 1 shows partial sequence from the C. albicans genome. Underlined regions were used to derive PCR primers, to generate a C. albicans ACC1 specific probe.

Figure 2 shows cloned fragments of the C. albicans ACC1 gene isolated from genomic DNA libraries. Arrows indicate extension of the fragment beyond the region displayed.

Figure 3 shows sequenced XbaI-HinDIII and HinDIII subclones of clone CLS1-b1.

Figure 4 shows the full DNA sequence of the C. albicans ACC1 gene. The atg start codons for Met1 and Met 2 are in lower case and underlined, as is the tag stop codon.

Figure 5 shows the full protein sequence of the C. albicans ACC1 gene. Putative start codons for Met1 and Met2 are shown in bold.

Figure 6 shows the generation of a tailored ACC1 gene (minus promoter) for expression under control of the GAL1 promoter in plasmid pYES2. From the initial ACCase gene (line1) the core SacI-BamHI (line3) is modified by the addition of 3' BamHI-NotI (line2) and 5' StuI-SacI (different fragments for Met1 and -2 lines 5 and 7 respectively) to generate the final "portable" gene flanked by StuI-NotI (lines 6 and 8).

Figure 7 shows the results of the *in-vitro* ACCase enzyme assay set out in the accompanying Example when Soraphen A (a specific inhibitor of the ACCase enzyme) was supplied (X-axis) over the range 0.1nM-100μM in the dose response regimen of the assay.

Example 1

Cloning of the C. albicans ACC1 gene and generation of a heterologous S. cerevisiae expression system:

1) Probe generation

We used the polymerase chain reaction (PCR) to generate a DNA probe between and including the underlined regions in Figure 1

2) Identification of clones from a C. albicans genomic library hybridising to the ACCase probe

The PCR product was labelled using an "ECL direct nucleic acid labelling and detection kit" (Amersham) as described by the supplier. The PCR product (probe) was then shown to hybridise to S. cerevisiae (weakly) and C. albicans genomic DNA. in a Southern blot procedure (as described Maniatis, 1989). Two genomic DNA libraries (CLS1 and CLS2) of C. albicans (in the yeast-E. coli shuttle plasmids YEp24 and pRS316 respectively, (as described in.Sherlock et al. 1994, source: Prof. John Rosamond, Manchester University) were used to isolate fragments hybridising with the probe which was radiolabelled using "Ready To Go" dCTP labelling beads (Pharmacia, as described by the manufacturer). The colony hybridisation was carried out as described by Maniatis (1989). Hybridising colonies were identified, plasmid DNA isolated, purified (Quiagen maxiprep, as described by the supplier) and sequenced (Applied Biosystems, model 377 sqeuencer) from their junctions with the plasmid. Several fragments carrying partial ACCase gene sequence as well as one full length clone could be identified (Figure 2).

3) Sequencing of the cloned gene, comparison with ACCases from S. cerevisiae, other fungi and higher eukaryotes (plants, mammals, man)

The bulk of the sequence of the C. albicans ACC1 gene was determined (on both strands) using flanking sequence- or insert sequence-specific primers from defined HinDIII and XbaI-HinDIII subfragments (of clone CLS1-b1) cloned into pUC19 (see Figure 4). The promoter and 5' coding region absent from this clone was established from CLS2-d1 and the gene's 3' end from CLS2-13 using insert specific primers. All junctions including the ones between the HinDIII subfragments were verified from the full length clone CLS2-13 (in Yep24. The full length DNA sequence of C. albicans (Ca) ACC1 is shown in Figure 5a and the protein translation in Figure 5b. The two potential start Methionines, Met1 and Met2 are shown in bold

The protein is homologous to ACCases of other fungi (S. cerevisiae, S. pombe and

U maydis) and also to the plant (Brassica napus), mammalian (sheep, chicken and rat) and human enzymes. Of the two potential start codons of C. albicans ACC1, Met 2 seems the more likely one as the sequence between Met1 and Met2 is unrelated to the other ACCases and indeed to any other protein sequence in the EMBL/Genbank database. The high degree of homology between ACCases of different species and the apparent lack of an identifiable fungal subgroup makes it even more important to use the actual target enzyme (here from the pathogen C. albicans) as a screening tool to identify specific inhibitors.

4) Generation of a heterozygous ACC1 deletion strain of S. cerevisiae

As ACCase is an essential enzyme, only one allele of a diploid cell can be deleted without loss of survival. One ACC1 gene of a diploid S. cerevisiae strain (JK9-3Daa, Kunz et al. 1993) was therefore disrupted using the kanamycin resistance cassette as described by Wach et al. using the protocol described therein. Sporulation of the heterozygous diploid (ACC1/acc1::KANMx) yields only two viable spores (which are kanamycin-sensitive) showing the essentiality of the ACC1 gene as well as the characteristic arrest phenotype for the two inviable spores (as published by Haßlacher et al., 1993).

5) Complementation of a S. cerevisiae ACC1 deletion with the cloned Candida gene, Ca ACC1

The heterozygous ACC1/acc1::KANMx strain was transformed with one full length C. albicans gene (CLS2-13 in Yep24). Expression of the gene from this plasmid will be due to functionality of the Candida ACC1 promoter in the heterologous S. cerevisiae system. Complementation of the knockout was demonstrated by sporulating the diploid transformants. In most cases 3-4 viable (haploid) spores were detected. The analysis of tetrads indicated that kanamycin-resistant colonies were only formed if they also contained the complementing CLS2-13 plasmid, as indicated by the presence of the URA3 transformation marker. This clearly shows that the C. albicans gene fully complements the ACCase function in S. cerevisiae. Therefore the strain generated can be used to screen for inhibitors which are specific for the Candida enzyme in the absence of a background of Saccharomyces enzyme.

As demonstrated by its functionality, the heterologous protein folds correctly in the host, S. cerevisiae, where it must also have been correctly biotinylated by the S. cerevisiae machinery (carried out by ACC2, encoding protein-biotin-ligase).

To facilititate purification of C. albicans ACCase, it is beneficial to achieve overexpression of the protein in a suitable host. Therefore the C. albicans promoter was replaced by the stronger and inducible S. cerevisiae GAL1 promoter. As the Candida sequence had revealed two potential start codons (see Figure 4) for the ACC1 reading frame, both versions were placed under GAL1 control. To generate appropriate restriction sites for cloning, the ACC1 gene was modified via PCR at both ends (see Figure 6 above). and cloned into plasmid pYES2 (Invitrogen) as a StuI-NotI fragment into HinDIII (fill-in)-NotI sites of the vector. The identity of the PCR-modified gene-parts with the original ones was confirmed by sequencing. Both constructs (Met1 and Met2) complement the S. cerevisiae ACC1 knockout when the cells are grown on galactose but not on glucose (where the GAL1 promoter is switched off). Growth is very poor if the gene is transcribed initiating at Met1, whereas Met2 restores wild type growth rates in S.cerevisiae.

6) Overexpression of the Ca ACCase to facilitate protein purification and use for screening purposes

Materials

Growth Media :-

Sabouraud Dextrose broth

Yeast peptone dextrose broth (YPD)

Yeast peptone galactose broth (YPGal) (i.e. 2% w/v galactose)

Growth of cells

Candida albicans B2630 (Janssen Pharmaceutica, Beerse, Belgium) was maintained on Sabouraud dextrose agar slopes at 37 ^{0}C which were subcultured biweekly. For the growth of liquid cultures for experiments, *C. albicans* grown on Sabourauds dextrose agar for

48 h at 37°C was used to inoculate 50 ml Sabouraud dextrose broth containing 500µg/l d-biotin. This was incubated for 16 h at 37 °C on a platform shaker (150 rpm). 1.5 ml of this culture was added to each of 24 x2 litre conical flasks, each containing 1 litre of Sabouraud dextrose broth containing 500µg/l d-biotin, giving a final inoculum concentration of approximately $1.5x10^6$ cfu ml^{-1}. The cultures were grown for 9 h, at 37 °C (log phase) with shaking (150 rpm). Cell numbers in liquid culture were determined spectrophotometrically (Philips PU8630 UV/VIS/NIR Spectrophotometer) at 540 nm in a 1 cm path length cuvette. Absorbance was linearly related to cell number up to an OD. of 2.0.

Saccharomyces cerevisiae strains Mey134 and CLS2-13 were maintained on Yeast peptone dextrose (YPD) agar plates at 30 °C, which were subcultured biweekly. For the growth of liquid cultures for experiments, the *S. cerevisiae* strains were grown on YPD agar for 48 h at 30 °C and were then used to inoculate 50 ml YPD broth containing 500µg/l d-biotin, which was incubated at 30°C for 16h on a platform shaker (200 rpm). 2.0 ml of this culture (approx. 4×10^8 cfu/ml) was added to each of 24 x 2 litre conical flasks, each containing 1 litre of YPD broth containing 500µg/l d-biotin, giving a final inoculum concentration of approximately $8 x10^5$ cfu/ml. The cultures were grown for 9 h, at 30 °C (log phase) with shaking (200 rpm). Cell numbers in liquid culture were determined spectrophotometrically (Philips PU8630 UV/VIS/NIR Spectrophotometer) at 540 nm in a 1 cm path length cuvette.

Saccharomyces cerevisiae strains PNS117a 5C, PNS117b 6A, and PNS 120a 6C were maintained on Yeast peptone galactose (YPGal) agar plates at 30 °C which were subcultured biweekly. For the growth of liquid cultures for experiments, the *S. cerevisiae* strains were grown on YPGal agar for 48 h at 30 °C and were then used to inoculate 50 ml YPGal broth containing 500µg/l d-biotin and 200µg/ml kanomycin, which were incubated at 30°C for 30h on a platform shaker (200 rpm). 2.0 ml of this culture (approx. 4×10^8 cfu/ml) was added to each of 24 x2 litre conical flasks, each containing 1 litre of YPGal broth containing 500µg/l d-biotin and 200µg/ml kanomycin, giving a final inoculum concentration of approximately 8

$x10^5$ cfu/ml. The cultures were grown for approximately 23h at 30 ^{0}C (log phase) with shaking (200 rpm).

Determination of cell number

Cell numbers were determined using a standard viable count agar based plating method, using the appropriate agar media.

Preparation of fungal ACCase enzyme

Cultures of the appropriate yeast strains were grown to the exponential phase of growth (for *Saccharomyces* and *Candida* strains respectively). These were then harvested by centrifugation (4400 g, 10min, 4 ^{0}C), washed twice in 700ml of 50mM Tris pH7.5 containing 20% w/v gylcerol, resuspending the cell pellet each time. The final washed pellet was fully resuspended into a thick slurry using 10 to 20ml of buffer (50mM Tris pH7.5 containing 1mM EGTA, 1mM EDTA (disodium salt), 1mM DTT, 0.25mM Pefabloc hydrochloride, 1µM Leupeptin hemisulphate, 1µM Pepstatin A, 0.5µM Trypsin inhibitor and 20% w/v glycerol). The volume of buffer required was dependent on the total packed cell wet weight. (i.e. 1ml buffer added per 6gm of packed wet cell pellet).

The cell paste was homogenised using a pre-cooled Bead-Beater (Biospec Products,Bartlesville, OK 74005) with 4 x 10 second Bursts, allowing 20 second intervals on ice. The preparation was then centrifuged at 31,180g for 30 minutes. After centrifugation the supernatant was immediately decanted into a container, then aliquoted before snap freezing in liquid nitrogen. The preparation was then stored at -80°C and was found to be stable for at least 2 months.

All enzyme preparation steps were carried out at +4°C, unless otherwise stated.

***In-vitro* ACCase enzyme assay**

The assay was conducted in 96 well, flat bottomed polystyrene microtitre plates. All test and control samples were tested in duplicate in this assay.

100µl of the ACCase enzyme preparation (in 50mM Tris pH7.5 containing 1mM EGTA, 1mM EDTA (disodium salt), 1mM DTT, 0.25mM Pefabloc hydrochloride, 1µM Leupeptin hemisulphate, 1µM Pepstatin A, 0.5µM Trypsin inhibitor, and 20% w/v glycerol) was added to each well of the microtitre plate. Each well contained either a 3µl test sample made up in DMSO or 3µl DMSO alone (NB. Final DMSO concentrations in the assay were 1.48% v/v). The microtitre plates were placed in a water bath maintained at 37°C. 10µl of [^{14}C] $NaHCO_3$ containing 9.25kBq in 378mM $NaHCO_3$ was then added to each well. The reaction was initiated by the addition of 100µl of Acetyl Coenzyme A containing assay buffer (50mM Tris pH7.5 containing 4.41mM ATP(disodium salt), 2.1mM Acetyl Coenzyme A, 2.52mM DTT, 10.5mM $MgCl_2$, and 0.21% w/v Albumin [Bovine, fraction V]), (removed from ice 5 minutes before use) to each well. The tubes were incubated at 37°C for 5 minutes. The reaction was then terminated by the addition of 50µl of 6M HCl to each well. In parallel, a pre-stopped assay control was set up which involved adding the 50µl of 6M HCl prior to [^{14}C] $NaHCO_3$ and the assay buffer (No further HCl additions were made to these wells after the 5 minute incubation). The DPM values for the pre-stopped assay were subtracted from the normal assay situation.

After the addition of the stop reagent the plates were left open in the water bath for a further 30 minutes to allow the $^{14}CO_2$ to escape. After this time 150µl of each reaction mixture were applied onto individual GF/C glass microfibre filter discs and allowed to dry thoroughly before adding scintillation fluid. Radioactivity in the samples was then determined by scintillation counting (Wallac WinSpectral 1414, Turku, Finland).

IC50's were calculated from the data using non-linear regression techniques available in the ORIGIN software package (Microcal Software Inc., Massachusetts, USA).

Soraphen A which is a specific inhibitor of ACCase was supplied over the range 0.1nM-100µM in the dose response regimen of the assay.

Protein determination

The total protein concentration of each ACCase preparation used was determined by the Coomasie Blue method (Pierce, Illinois, USA), (using 1cm path length cuvettes read 595nm (Philips PU8630 UV/VIS/NIR Spectrophotometer).

In-vitro antifungal activity

Compounds were tested over a concentration range of 1024 - 0.00098 µg/ml by a broth-dilution method in microtitre plates using doubling dilutions in YPD or YPGal (both containing 500µg/l d-biotin). Stock solutions of inhibitors were prepared at 51.2mg/ml in Dimethyl sulphoxide (DMSO) (final assay concentration of DMSO was 2% v/v). Each Yeast culture was added to the well to give a final 10^4 cfu/well. The plates were incubated at 30°C for 48h and MIC's determined visually.

Discussion

Expression of ACCase, a biotinylated protein, was monitored by a "biotin-avidin affinity western blot" as described by Haßlacher et al., 1993. Expression of the C. albicans ACC1 gene from its own promoter from plasmid Yep24 was comparable to that of the S. cerevisiae gene (no overexpression). Expression under control of the GAL1 promoter however, was considerably higher indicating a drastically increased level of biotinylated and therefore fully functional enzyme. Transcription of the gene was fully induced as the cells had to be grown on galactose to be viable. On glucose the GAL1 promoter is completely off, causing the cells to arrest and eventually die due to insufficient supply of ACCase). The S. cerevisiae strain described in this application is a convenient source of the C. albicans enzyme. The engineered strain possesses no residual background ACCase because the gene coding for the S. cerevisiae enzyme had been removed. Congenic versions of such a strain (genetically identical apart from the ACCase gene carried) expressing different ACCases (e.g. the different human (Abu-Elheiga et al. 1995), mammalian (Lopez-Casillas et al., 1988, Takai et al. 1988, Barber et al., 1995)), plant (Schulte et al., 1994) or other fungal enzymes (Al-Feel et al., 1992, Saito et al., 1996, Bailey et al., 1995)) can be used as tools for

screening. Differences in growth of such strains may be solely dependent on differences in their ACCase activity. Differential growth in the presence of ACCase inhibitors (for example soraphenA or compounds yet to be identified) indicates selectivity of the drug towards one type of the ACCase enzyme.

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TABLE 1

Comparative properties of native and recombinant acetyl-CoA carboxylase enzymes

Yeast strain	Cell doubling time (minutes)	Growth temperature for ACCase preparation (°C)	Liquid MIC (µg/ml) for Soraphen A	IC50 for Soraphen A (nM) against ACCase preparations	Specific activity of ACCase preparation (nmoles product/ min/mg protein)
C. albicans B2630	56	37	0.003		
S. cerevisiae Mey 134	160	30	8		0.641
S. cerevisiae CLS2-13	163	30	2	2.499	3.054
S. cerevisiae PNS 117a 5C	253	30	2	17.518	7.025
S. cerevisiae PNS 117b 6A	222	30	4	13.083	10.573
S. cerevisiae PNS 120a 6C	303	30	0.5	ND	0.244
S. cerevisiae PNS 120b 1C	287	30	0.125	ND	ND

Key :- ND = not determined

Claims:

1. A polynucleotide encoding an Acetyl-COA-carboxylase (ACCase) gene from *Candida albicans*.

2. A polynucleotide as claimed in claim 1 and as set out in Figure 4 herein.

3. A polynucleotide as claimed in claim 2 and characterised by the start codon atg2.

4. A polynucleotide comprising a restriction fragment of a polynucleotide as claimed in any one of claims 1-3.

5. A polynucleotide probe comprising a polynucleotide as claimed in any one of claims 1-4.

6. An Acetyl-COA-carboxylase (ACCase) polypeptide from *Candida albicans* in isolated and purified form.

7. A polypeptide as claimed in claim 6 and as set out in Figure 5.

8. A polypeptide as claimed in claim 7 and characterised by Met2.

9. A polypeptide as claimed in claim 6 and obtained by expression of a polynucleotide as claimed in any one of claims 1-4.

10. Antibodies specific for a polypeptide as claimed in any one of claims 6-9.

11. An antisense polynucleotide specific for all or a part of a polynucleotide as claimed in any one of claims 1-4.

12. An RNA transcript corresponding to a polynucleotide as claimed in any one of claims 1-4.

13. An expression system for expression of an Acetyl-COA-carboxylase (ACCase) polypeptide from *Candida albicans* which system comprises an *S. cerevisiae* host strain having a *Candida albicans* ACC1 polynucleotide as claimed in any one of claims 1-3, inserted in place of the native ACC1 gene from *S. Cerevisiae*, whereby the *Candida albicans* ACC1 polypeptide is expressed.

14. An expression system as claimed in claim 13 and adapted for controlled overexpresssion of the *Candida albicans* polynucleotide relative to expression under the control of a *Candida albicans* promoter

15. An expression system as claimed in claim 14 and used to provide an Acetyl-COA-carboxylase (ACCase) gene from *Candida albicans* in sufficient quantity and with sufficient activity for compound screening purposes.

16. Use of an Acetyl-COA-carboxylase (ACCase) polypeptide from *Candida albicans* as claimed in claim 6, in an assay to identify inhibitors of the polypeptide.

17. Use as claimed in claim 16 in pharmaceutical research.

FIGURE 1

GCACGCTTGACGGTTTTCACCAAATGCGAAAATATGACCAAATTGAGAATCCGAAAATGA
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TTCTGACTTGAATTCAAAATCAATATCAGTAGTGGTATGAGGATCGGCACCGCACAAAGT
TCTGATATCTCTGATTCTATGCATTGGTATACCCATAGCAATTTGTAATTGAGCAGCTGG
TAAATTAACACCTGTCACCATTTCAGTGGTTGGATGTTCAACTTGCAATCTTGGGTTCAA
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FIGURE 2

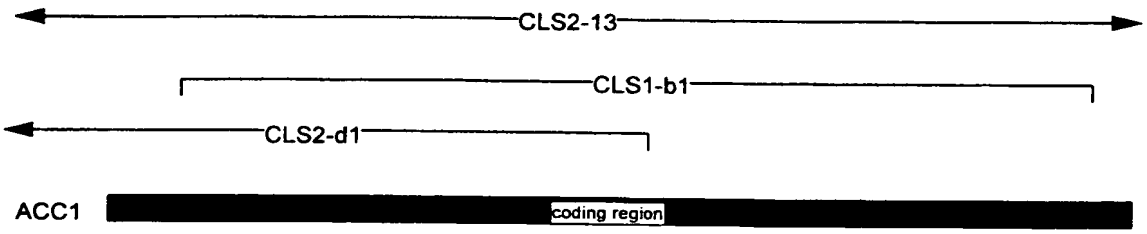

FIGURE 3

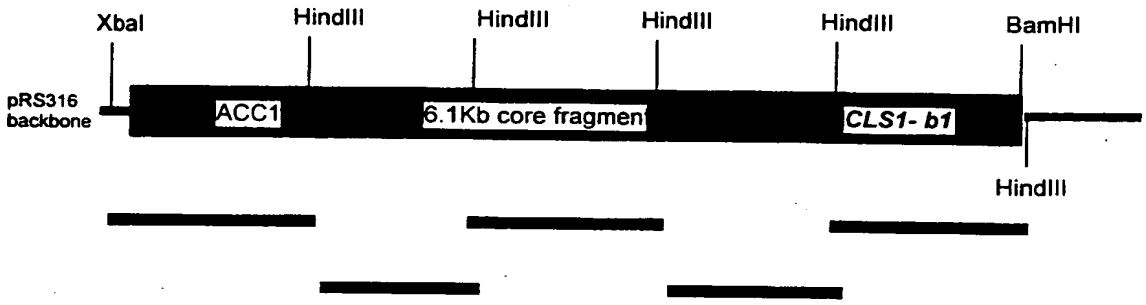
XbaI
HindIII
HindIII
HindIII
HindIII
BamHI
pRS316 backbone
ACC1
6.1Kb core fragmen
CLS1- b1
HindIII

FIGURE 4

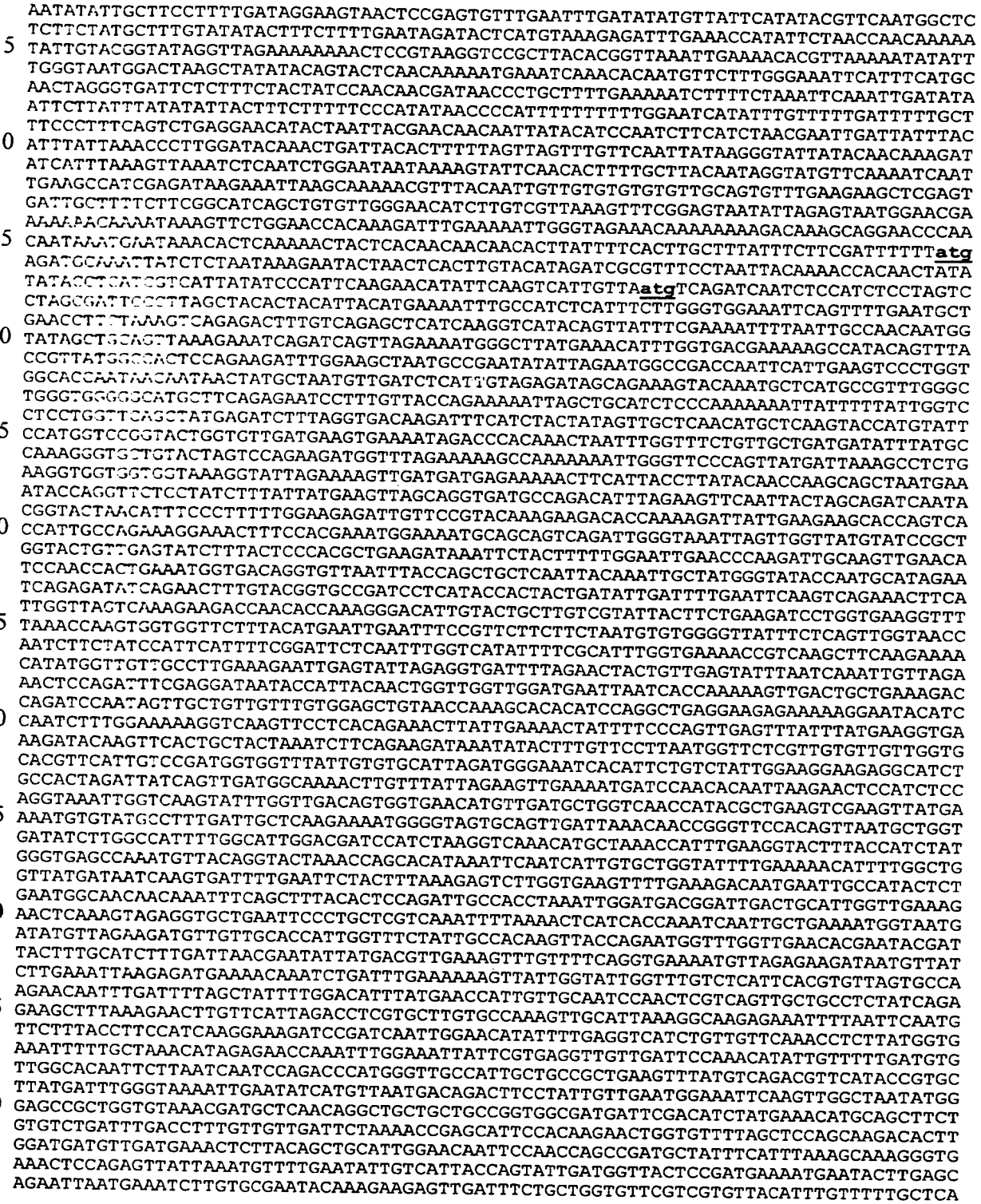

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FIGURE 5

MRCKLSLIKNTNSLVHRSRFLITKPQLYIPHRHYIPFKNIFKSLLMSDQSPSPSPSDSLSYTTLHENLPSHFLGGNSVLN
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FIGURE 6

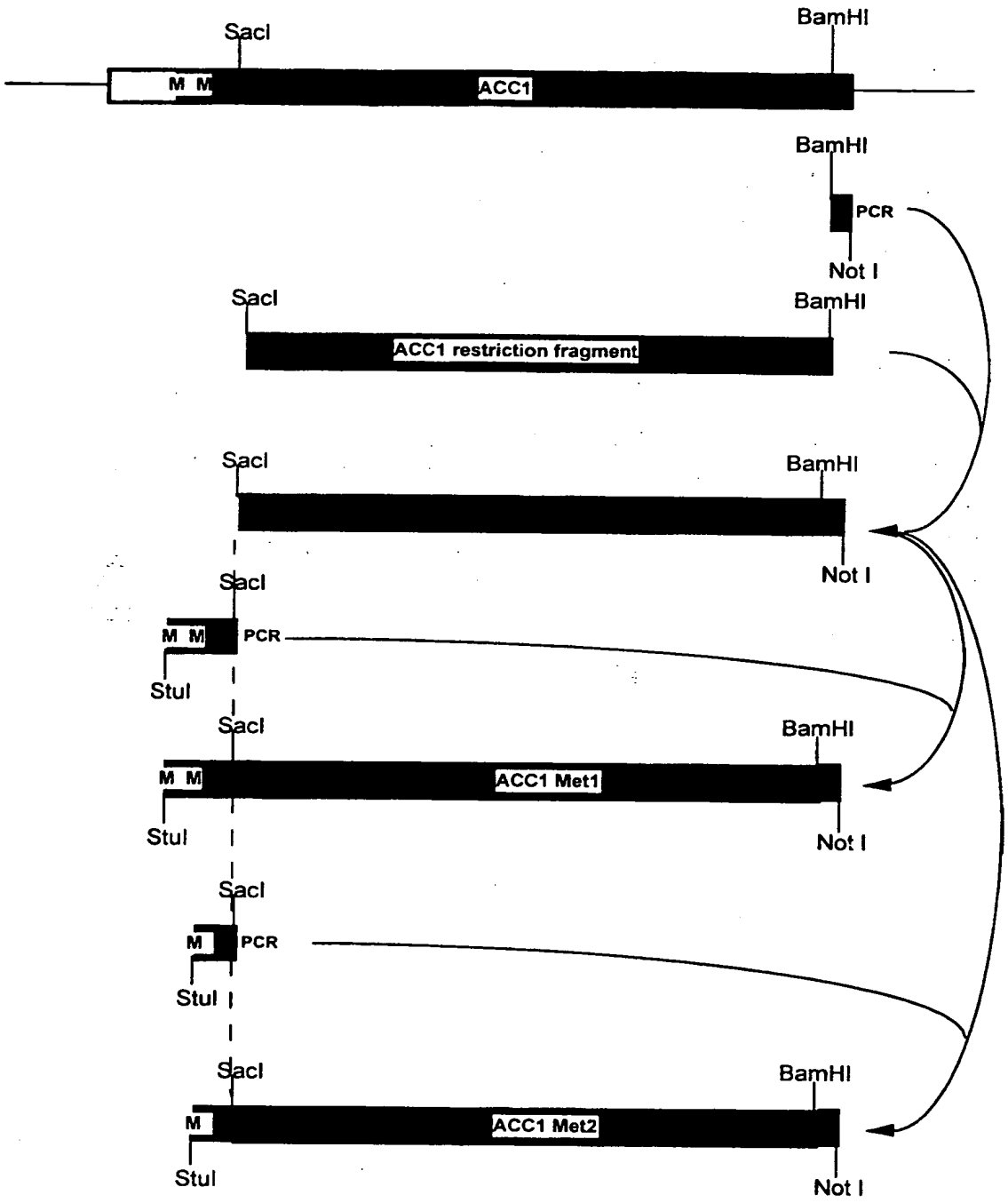
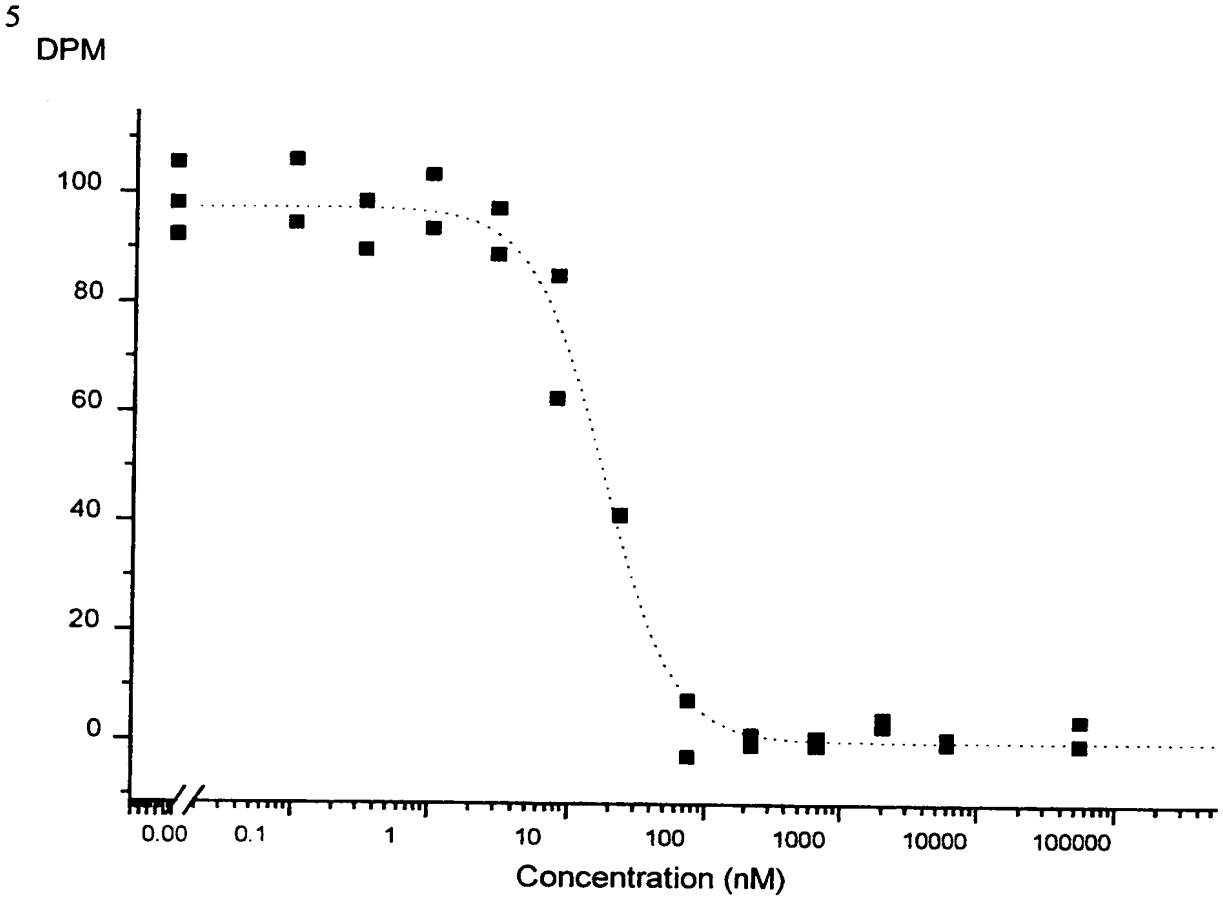


FIGURE 7



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(C) CITY: London
(D) STATE: Greater London
(E) COUNTRY: England
(F) POSTAL CODE (ZIP): W1Y 6LN
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(I) TELEX: 0171 834 2042

(ii) TITLE OF INVENTION: PROCESS

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9726897.3
(B) FILING DATE: 20-DEC-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 523 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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CATTAATTCA ACTTTTTAAT GACATTGAAA GTAGTAGTAG TTGTTGTTTT TTAGATTTAA 7920
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AGAGTGGGAA ATTGGCTTTT TTGTTGCTCG TGTGATGAAA CAGTGATTGA CACAAAAAAA 8040
TAGACAATGA AAAC 8054

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2270 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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            20                  25                  30
His Tyr Ile Pro Phe Lys Asn Ile Phe Lys Ser Leu Leu Met Ser Asp
        35                  40                  45
Gln Ser Pro Ser Pro Ser Pro Ser Asp Ser Leu Ser Tyr Thr Thr Leu
    50                  55                  60
His Glu Asn Leu Pro Ser His Phe Leu Gly Gly Asn Ser Val Leu Asn
65                  70                  75                  80
Ala Glu Pro Ser Lys Val Arg Asp Phe Val Arg Ala His Gln Gly His
                85                  90                  95
Thr Val Ile Ser Lys Ile Leu Ile Ala Asn Asn Gly Ile Ala Ala Val
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Glu Lys Ala Ile Gln Phe Thr Val Met Ala Thr Pro Glu Asp Leu Glu
    130                 135                 140
Ala Asn Ala Glu Tyr Ile Arg Met Ala Asp Gln Phe Ile Glu Val Pro
145                 150                 155                 160
Gly Gly Thr Asn Asn Asn Asn Tyr Ala Asn Val Asp Leu Ile Val Glu
                165                 170                 175
Ile Ala Glu Ser Thr Asn Ala His Ala Val Trp Ala Gly Trp Gly His
            180                 185                 190
Ala Ser Glu Asn Pro Leu Leu Pro Glu Lys Leu Ala Ala Ser Pro Lys
        195                 200                 205
Lys Ile Ile Phe Ile Gly Pro Pro Gly Ser Ala Met Arg Ser Leu Gly
    210                 215                 220
Asp Lys Ile Ser Ser Thr Ile Val Ala Gln His Ala Gln Val Pro Cys
225                 230                 235                 240
Ile Pro Trp Ser Gly Thr Gly Val Asp Glu Val Lys Ile Asp Pro Gln
                245                 250                 255
Thr Asn Leu Val Ser Val Ala Asp Asp Ile Tyr Ala Lys Gly Cys Cys
            260                 265                 270
Thr Ser Pro Glu Asp Gly Leu Glu Lys Ala Lys Lys Ile Gly Phe Pro
        275                 280                 285
Val Met Ile Lys Ala Ser Glu Gly Gly Gly Gly Lys Gly Ile Arg Lys
    290                 295                 300
Val Asp Asp Glu Lys Asn Phe Ile Thr Leu Tyr Asn Gln Ala Ala Asn
305                 310                 315                 320
Glu Ile Pro Gly Ser Pro Ile Phe Ile Met Lys Leu Ala Gly Asp Ala
                325                 330                 335
Arg His Leu Glu Val Gln Leu Leu Ala Asp Gln Tyr Gly Thr Asn Ile
            340                 345                 350
Ser Leu Phe Gly Arg Asp Cys Ser Val Gln Arg Arg His Gln Lys Ile
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Ile Glu Glu Ala Pro Val Thr Ile Ala Arg Lys Glu Thr Phe His Glu
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385                 390                 395                 400
Ala Gly Thr Val Glu Tyr Leu Tyr Ser His Ala Glu Asp Lys Phe Tyr
                405                 410                 415
Phe Leu Glu Leu Asn Pro Arg Leu Gln Val Glu His Pro Thr Thr Glu
            420                 425                 430
Met Val Thr Gly Val Asn Leu Pro Ala Ala Gln Leu Gln Ile Ala Met
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Gly Ile Pro Met His Arg Ile Arg Asp Ile Arg Thr Leu Tyr Gly Ala
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Asp Pro His Thr Thr Thr Asp Ile Asp Phe Glu Phe Lys Ser Glu Thr
465                 470                 475                 480
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Cys Arg Ile Thr Ser Glu Asp Pro Gly Glu Gly Phe Lys Pro Ser Gly
500 505 510
Gly Ser Leu His Glu Leu Asn Phe Arg Ser Ser Ser Asn Val Trp Gly
515 520 525
Tyr Phe Ser Val Gly Asn Gln Ser Ser Ile His Ser Phe Ser Asp Ser
530 535 540
Gln Phe Gly His Ile Phe Ala Phe Gly Glu Asn Arg Gln Ala Ser Arg
545 550 555 560
Lys His Met Val Val Ala Leu Lys Glu Leu Ser Ile Arg Gly Asp Phe
565 570 575
Arg Thr Thr Val Glu Tyr Leu Ile Lys Leu Leu Glu Thr Pro Asp Phe
580 585 590
Glu Asp Asn Thr Ile Thr Thr Gly Trp Leu Asp Glu Leu Ile Thr Lys
595 600 605
Lys Leu Thr Ala Glu Arg Pro Asp Pro Ile Val Ala Val Val Cys Gly
610 615 620
Ala Val Thr Lys Ala His Ile Gln Ala Glu Glu Glu Lys Lys Glu Tyr
625 630 635 640
Ile Gln Ser Leu Glu Lys Gly Gln Val Pro His Arg Asn Leu Leu Lys
645 650 655
Thr Ile Phe Pro Val Glu Phe Ile Tyr Glu Gly Glu Arg Tyr Lys Phe
660 665 670
Thr Ala Thr Lys Ser Ser Glu Asp Lys Tyr Thr Leu Phe Leu Asn Gly
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Ser Arg Cys Val Val Gly Ala Arg Ser Leu Ser Asp Gly Gly Leu Leu
690 695 700
Cys Ala Leu Asp Gly Lys Ser His Ser Val Tyr Trp Lys Glu Glu Ala
705 710 715 720
Ser Ala Thr Arg Leu Ser Val Asp Gly Lys Thr Cys Leu Leu Glu Val
725 730 735
Glu Asn Asp Pro Thr Gln Leu Arg Thr Pro Ser Pro Gly Lys Leu Val
740 745 750
Lys Tyr Leu Val Asp Ser Gly Glu His Val Asp Ala Gly Gln Pro Tyr
755 760 765
Ala Glu Val Glu Val Met Lys Met Cys Met Pro Leu Ile Ala Gln Glu
770 775 780
Asn Gly Val Val Gln Leu Ile Lys Gln Pro Gly Ser Thr Val Asn Ala
785 790 795 800
Gly Asp Ile Leu Ala Ile Leu Ala Leu Asp Asp Pro Ser Lys Val Lys
805 810 815
His Ala Lys Pro Phe Glu Gly Thr Leu Pro Ser Met Gly Glu Pro Asn
820 825 830
Val Thr Gly Thr Lys Pro Ala His Lys Phe Asn His Cys Ala Gly Ile
835 840 845
Leu Lys Asn Ile Leu Ala Gly Tyr Asp Asn Gln Val Ile Leu Asn Ser

850 855 860

Thr Leu Lys Ser Leu Gly Glu Val Leu Lys Asp Asn Glu Leu Pro Tyr
865 870 875 880

Ser Glu Trp Gln Gln Gln Ile Ser Ala Leu His Ser Arg Leu Pro Pro
885 890 895

Lys Leu Asp Asp Gly Leu Thr Ala Leu Val Glu Arg Thr Gln Ser Arg
900 905 910

Gly Ala Glu Phe Pro Ala Arg Gln Ile Leu Lys Leu Ile Thr Lys Ser
915 920 925

Ile Ala Glu Asn Gly Asn Asp Met Leu Glu Asp Val Val Ala Pro Leu
930 935 940

Val Ser Ile Ala Thr Ser Tyr Gln Asn Gly Leu Val Glu His Glu Tyr
945 950 955 960

Asp Tyr Phe Ala Ser Leu Ile Asn Glu Tyr Tyr Asp Val Glu Ser Leu
965 970 975

Phe Ser Gly Glu Asn Val Arg Glu Asp Asn Val Ile Leu Lys Leu Arg
980 985 990

Asp Glu Asn Lys Ser Asp Leu Lys Lys Val Ile Gly Ile Gly Leu Ser
995 1000 1005

His Ser Arg Val Ser Ala Lys Asn Asn Leu Ile Leu Ala Ile Leu Asp
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1075 1080 1085

Val Gln Thr Ser Tyr Gly Glu Ile Phe Ala Lys His Arg Glu Pro Asn
1090 1095 1100

Leu Glu Ile Ile Arg Glu Val Val Asp Ser Lys His Ile Val Phe Asp
1105 1110 1115 1120

Val Leu Ala Gln Phe Leu Ile Asn Pro Asp Pro Trp Val Ala Ile Ala
1125 1130 1135

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1155 1160 1165

Phe Lys Leu Ala Asn Met Gly Ala Ala Gly Val Asn Asp Ala Gln Gln
1170 1175 1180

Ala Ala Ala Ala Gly Gly Asp Asp Ser Thr Ser Met Lys His Ala Ala
1185 1190 1195 1200

Ser Val Ser Asp Leu Thr Phe Val Val Asp Ser Lys Thr Glu His Ser
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1315 1320 1325
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1345 1350 1355 1360
Ile His Val Tyr Asp Ala Ile Gly Lys Asn Ala Pro Ser Asp Lys Arg
1365 1370 1375
Phe Phe Thr Arg Gly Ile Ile Arg Thr Gly Val Leu Lys Glu Asp Ile
1380 1385 1390
Ser Ile Ser Glu Tyr Leu Ile Ala Glu Ser Asn Arg Leu Met Asn Asp
1395 1400 1405
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Val Glu Ala Ala Phe Gly Ser Phe Leu Glu Arg Phe Gly Arg Arg Leu
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1460 1465 1470
Pro Gln Gly Thr Ser Phe Pro Leu Arg Ala Ile Ile Asn Asn Val Ser
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1490 1495 1500
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Lys Val Pro Lys Asp Val Phe Val Ser Leu Glu Leu Ile Thr Asp Glu
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1845 1850 1855
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1925 1930 1935
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1940 1945 1950
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1955 1960 1965
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1970 1975 1980

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2005 2010 2015
Val Asp Phe Lys Gln Pro Ile Phe Thr Tyr Ile Pro Pro Asn Gly Glu
2020 2025 2030
Leu Arg Gly Gly Ser Trp Val Val Val Asp Pro Thr Ile Asn Ser Asp
2035 2040 2045
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2065 2070 2075 2080
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2100 2105 2110
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2245 2250 2255
Leu Ser Lys Glu Asp Lys Glu Lys Phe Leu Lys Ala Leu Lys
2260 2265 2270

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/GB 98/03857

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/52 C12N9/00 C07K16/40 C12N15/11 C12N15/81
C12Q1/25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	FR 2 727 129 A (RHONE POULENC AGROCHIMIE) 24 May 1996 see the whole document ---	1-13,16, 17 14,15
X	AL-FEEL W ET AL: "Cloning of the yeast FAS3 gene and primary structure of yeast acetyl-CoA carboxylase" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 89, May 1992, pages 4534-4538, XP002097900 WASHINGTON US see the whole document ---	1-12
Y	GB 2 137 208 A (COLLABORATIVE RES INC) 3 October 1984 see the whole document --- -/--	14,15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 March 1999	09/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van der Schaal, C

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	HORIKAWA S ET AL: "CELL-FREE TRANSLATION AND REGULATION OF CANDIDA -LIPOLYTICA ACETYL COENZYME A CARBOXYLASE EC-6.4.1.2 MESSENGER RNA." EUR J BIOCHEM, (1980) 104 (1), 191-198. CODEN: EJBCAI. ISSN: 0014-2956., XP002097901	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna[redacted]cation No
PCT/GB 98/03857

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2727129 A	24-05-1996	NONE	
GB 2137208 A	03-10-1984	US 4661454 A	28-04-1987
		AT 64416 T	15-06-1991
		CA 1283373 A	23-04-1991
		CA 1273883 C	11-09-1990
		DK 97784 A	29-08-1984
		EP 0123811 A	07-11-1984
		JP 60058077 A	04-04-1985
		US 5139936 A	18-08-1992